Pulmonary Pharmacology & Therapeutics 25 (2012) 447–452

Contents lists available at SciVerse ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

Respiratory epithelial cell responses to cigarette smoke: The unfolded protein response

Steven G. Kelsen*

Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Temple University School of Medicine, USA

ARTICLE INFO

Article history:
Received 2 March 2012
Received in revised form
28 June 2012
Accepted 20 July 2012

Keywords:
Protein metabolism
Protein misfolding
Endoplasmic reticulum stress

ABSTRACT

Cigarette smoking exposes the respiratory epithelium to highly toxic, reactive oxygen nitrogen species which damage lung proteins in the endoplasmic reticulum (ER), the cell organelle in which all secreted and membrane proteins are processed. Accumulation of damaged or misfolded proteins in the ER, a condition termed ER stress, activates a complex cellular process termed the unfolded protein responses (UPR). The UPR acts to restore cellular protein homeostasis by regulating all aspects of protein metabolism including: protein translation and syntheses; protein folding; and protein degradation. However, activation of the UPR may also induce signaling pathways which induce inflammation and cell apoptosis. This review discusses the role of UPR in the respiratory epithelial cell response to cigarette smoke and the pathogenesis of lung diseases like COPD.

1. Introduction

Cigarette smoking, the primary cause of chronic obstructive lung disease (COPD) and lung cancer, exposes the airway epithelium to a variety of deleterious substances. In fact, smoke from a burning cigarette contains more than 4500 separate compounds many of which are highly toxic reactive oxygen/nitrogen species (RONS) and xenobiotic materials [1–4] Although the molecular mechanisms underlying lung and airway damage in response to cigarette smoke remain incompletely understood, RONS are believed to adversely affect oxidant/anti-oxidant and protease/anti-protease balance in the lung thereby inciting an inflammatory reaction and killing cells [2,5–10].

Recent studies by ourselves [11] and others [12–19] indicate that acute and chronic exposure to cigarette smoke adversely affects protein metabolism in the lung and lung epithelial cells. Specifically, cigarette smoke causes misfolding of nascent proteins in the lumen of the endoplasmic reticulum (ER), the cell organelle in which virtually all membrane and secreted proteins achieve their folded structure and configuration [11,16–19]. Moreover, cigarette smoke induces the accumulation of protein aggregates intracellularly and promotes autophagy, a cellular response designed to eliminate large amounts of terminally misfolded proteins [13,20]. Since misfolded proteins are non-functional and may even be cytotoxic, this effect of cigarette smoke is of potential importance in the pathogenesis of cigarette smoke-induced lung cell death and lung disease such as chronic obstructive pulmonary disease (COPD) amounts [13,21–24]. Fortunately, eukaryotic cells have evolved a biologically highly conserved compensatory response designed to reverse the accumulation of misfolded proteins in the endoplasmic reticulum (ER), termed the unfolded protein response (UPR) [23–7]. This review will discuss the effects of cigarette smoke on the UPR and its potential role in the development of cigarette smoke-induced lung disease.

2. The unfolded protein response (UPR)

The endoplasmic reticulum (ER) of eukaryotic cells is an extensive network of vesicles, cisternae and tubules. This organelle performs a variety of functions including production of membrane and secretory proteins, lipids and sterols as well as calcium storage and release. Nascent polypeptides which are transported in an unfolded form to the lumen of the ER from the ribosomal complex are modified to achieve their final 3 dimensional structure [26–30]. Proper protein folding requires an elaborate system of ER chaperones, foldases, isomerases, and oxido-reductases including ER resident chaperones (e.g., GRP78/calreticulin/calnexin/GRP94 and

* Room 761 Parkinson Pavilion, Temple University Hospital, 3401 North Broad Street, Philadelphia, PA 19140, USA. Tel.: +1 215 707 2237; fax: +1 215 707 6867.
E-mail address: kelsen@temple.edu.

1094-5539/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.pupt.2012.07.005

GRP 170) and foldases/isomerases (e.g., PDI [protein disulfide isomerase]).

ER stress occurs when the capacity to fold, modify and transport mature proteins cannot keep pace with the rate of protein synthesis and can be induced by exogenous organic and inorganic oxidants, RONS, decreases in ER calcium or hypoxia, all of which impair protein folding in the lumen of the endoplasmic reticulum (ER). RONS, in particular, may act by interfering with protein folding directly by oxidizing thiol groups or indirectly by impairing ER calcium homeostasis [28,31,32].

Protein folding in the ER lumen is monitored by a triad of ER resident ER stress sensors [i.e., PERK; ATF6 (activating transcription factor 6); and IRE1 (inositol requiring enzyme-1)] [26–30]. Although the precise mechanism by which an increase in the load of unfolded proteins is sensed is uncertain, dissociation of an inhibitor protein, the chaperone, GRP78, from the ER luminal surface of the sensors induces dimerization and auto-phosphorylation of the sensors thereby activating them and triggering a UPR. The UPR is a highly conserved, compensatory response, which relieves ER stress by affecting all aspects of protein metabolism. The UPR restores ER homeostasis in several ways: 1) by globally reducing protein synthesis; 2) by increasing the expression of proteins involved in the folding process; 3) by transporting terminally misfolded proteins for degradation by the proteasome or autophagosome; and 4) by expanding ER volume and capacity [33].

PERK is a transmembrane kinase which phosphorylates and thereby inhibits eIF2α (i.e., eukaryotic translation initiation factor-2α). Phosphorylation of eIF2α is a crucial feature of the UPR. Its phosphorylation inhibits protein translation globally, but facilitates translation of selected mRNAs. Specifically, phospho-eIF2α inhibits eIF2B, which catalyzes the exchange of GDP for GTP thereby diminishing the efficiency of ribosomal scanning. Facilitated translation as a result of eIF2α phosphorylation occurs in mRNAs which contain open reading frames 5′ upstream of coding regions which are not usually translated when ribosomal scanning efficiency is normal. Translation of ATF4, a member of the ATF/CREB family of transcription factors which controls the transcription of anti-oxidant genes (see below) is facilitated by eIF2α phosphorylation. ATF4 mRNA contains two upstream open reading frames, uORF1 and uORF2, located 50 BP upstream of the normally untranslated ATF4 coding sequence. In the absence of eIF2α phosphorylation, uORF1 and uORF2 are translated to the exclusion of ATF4 itself. The uORF2 overlaps with that for ATF4, but is out of frame. As a consequence of eIF2α phosphorylation, ribosome scanning bypasses uORF2, and translation re-initiation occurs at the ATF4 coding region. Thus, synthesis of ATF4 protein is selectively elevated in response to ER stress.

ATF4 also induces expression of the transcription factor, CHOP, [C/EBP-homologous protein] which induces cell apoptosis [18,19,21,27,34,35]. CHOP, in turn, inhibits expression of the anti-apoptotic factor, BCL-2, down-regulates the cell cycle transition regulator, p21, and activates executioner caspase 3 [21,27,34,35]. The precise mechanisms which regulate the balance between pro- and anti-apoptotic actions of the UPR are not well understood.

IRE1α and β, transmembrane kinases with RNAse activity, splice XBP1 mRNA into a transcription factor (sXBP1) which functions cooperatively with the transcription factor, ATF6 (see below), to up-regulate the above ER resident chaperones, as well as genes involved in protein ubiquitination and degradation and expansion of ER mass by increasing proteins involved in cholesterol metabolism and membrane synthesis [22,35–39]. IRE1 also inhibits protein synthesis by promiscuous degradation of various mRNAs via its RNAase activity, a process termed regulated-IRE1-dependent decay (RIDD), and induces apoptosis through its kinase activity by activating JNK, AP1 and NF-kB [21,40]. Of note, the IRE1β isoform is preferentially expressed in the lung and GI tract [23,41].

ATF6 is a proto-transcription factor, which upon proteolytic cleavage in the Golgi, traffics to the nucleus and in conjunction with spliced XBP1, activates genes encoding GRP78, calreticulin, calnexin, and PDI [39].

A schematic representation of the mechanism of activation of the 3 ER sensors and their downstream signaling pathways which mediate the UPR is provided in Fig. 1.

In general, activation of the UPR is indicated by up-regulation at the protein or mRNA level of the downstream molecules activated by PERK, IRE1 or ATF6 [42]. Generally, these are the chaperones, GRP78, calreticulin, calnexin and the foldase, PDI [protein disulfide isomerase], which reflect the activity of ATF6 and sXBP1. UPR activation has also been assessed by measurement of cleaved ATF6 protein and by sXBP1 mRNA [21,43,44].

3. Cigarette smoke induces an unfolded protein response in human airway epithelial cells and in the lungs of chronic smokers

We [11] and others [16–19] have shown that acute exposure of cultured human airway epithelial cells or fibroblasts (3T3 cells) activates the UPR. Activation of the UPR is reflected in phosphorylation of eIF2α, increases in ATF4, CHOP and ATF6 and up-regulation of the hallmark UPR effectors, GRP78, calreticulin, calnexin and PDI. Of interest, cigarette smoke preferentially activates the PERK and ATF6 arms of the UPR and may even inhibit the IRE1 arm [17]. In cultured cells, activation of the UPR by cigarette smoke is rapid (minutes) and dose dependent [11,18].

The lungs of chronic cigarette smokers also demonstrate an activated UPR as reflected by up-regulation at the protein level of the UPR chaperones, GRP78, calreticulin and calnexin [11],. Moreover, GRP78 expression is increased in small airway epithelial cells and type II pneumocytes of chronic cigarette smokers as assessed by immunohistochemistry (Fig. 2). Of interest, expression of GRP78, calreticulin and PDI is significantly lower in ex-smokers compared to smokers suggesting that the process is at least partially reversible with smoking cessation.

Several downstream pathways controlled by the UPR are also differentially expressed in the lungs of chronic smokers [11]. These include up-regulation of the anti-oxidant enzyme, thioredoxin-dependent peroxidase reductase; and several components of the polyribosome which regulate protein translation, i.e., elongation factor-1β, elongation factor-1δ, 60S acidic ribosomal protein P2 and heat shock protein 27.

4. Mechanisms of cigarette smoke-induced UPR

The mechanism(s) by which cigarette smoke induces an unfolded protein response is not entirely clear. Given that cigarette smoke contains a variety of inorganic and organic RONS, a direct oxidant oxidation of target proteins would appear to explain induced ER stress and protein misfolding. In fact, activation of the UPR is explainable, in part, by the action of superoxide and peroxynitrate RONS species since the use of appropriate free radical scavengers against these species and anti-oxidants inhibited the effect of cigarette smoke [18,19]. Of interest, the vapor phase of cigarette smoke phase appears to be more potent than the particulate phase in this regard perhaps because of a greater concentration of RONS [17]. In addition, the cigarette smoke vapor phase ingredient, acrolein per se, activates the UPR [16]. Cigarette smoke also increases cytosolic calcium suggesting the possibility that depletion of ER calcium may contribute to the development of ER stress and a UPR [45].